Three varieties of methyl citrate and 1-methyl malate were isolated from the fruits of *Opuntia ficus-indica* var. saboten Makino through *in vitro* bioassay-guided isolation for the inhibition on monoamine oxidase (MAO). The IC₅₀ values for MAO-B of 1-monomethyl citrate, 1,3-dimethyl citrate, trimethyl citrate and 1-methyl malate were 0.19, 0.23, 0.61 and 0.25 mM, respectively. However, on MAO-A, their inhibitions showed only marginal activity.

**Key words:** *Opuntia ficus-indica* var. saboten, Monoamine oxidase-B inhibitor, Citric acid methyl ester, 1-Methyl malate

**INTRODUCTION**

*Opuntia ficus-indica* var. saboten Makino is a tropical or subtropical plant (Cactaceae), which was introduced to seaside area of Cheju Island, Korea. Its fruits and stem have been used on Cheju Island as folk medicines for burns, edema and indigestion. Currently, it is cultivated on Cheju for use in manufacturing health foods such as tea, drinks, and noodles. For utilization as a food material, the color stability of the fruit (Kim et al., 1995; Chung et al., 1996), their composition (Lee et al., 1997), and the quality of wet noodles using its powder (Lee et al., 1999) have been previously investigated.

Several compounds from the cactus have been isolated. Piscidic acid, indicaxanthin in the fruits (Impellizzeri et al., 1972), oligosaccharides in the partially hydrolyzed mucilage (Mcgravie et al., 1981), neobetanin as a minor constituent in the petals (Alard et al., 1985) and two flavonols in the fruits (Jeong et al., 1999) were found in the cactus.

Recently, the ethanol extract of its stem was proven to show anti-inflammatory and analgesic actions in carrageenan-induced paw edema test in rats and acetic acid-induced writhing test in mice, respectively (Park et al., 1998). However, an active principle for the actions was not isolated.

In order to isolate pharmacologically active constituents from the cactus, we screened several bioassays including antithrombotic, anticoagulant, dopamine β-hydroxylase and monoamine oxidase (MAO) activities. Among these, it was found that the fruits and stem of the cactus inhibit MAO activity. This paper addresses the isolation of well known-but not previously reported in this plant-organic acid methylesters that show inhibitory activity against MAO-B from the methanol extract of fruits.

**MATERIALS AND METHODS**

**General experimental procedures**

Melting points were determined with a Mitamura-Riken melting point apparatus and were uncorrected. IR spectra were recorded in KBr using a Jasco FT/IR-5300 spectrophotometer. ¹H- and ¹³C-NMR spectra were obtained in CD₃OD or in CDCl₃ on a Varian Gemini 2000 spectrometer operating at 300 and 75.5 MHz, respectively. The chemical shifts were reported in parts per million, and the coupling constants (J values) were in Hertz. MS measurement was performed on a Hewlett Packard 5989B Mass spectrometer. TLC analyses were carried out on precoated silica gel F₂₅₄ plates (E. Merck, Darmstadt). The adsorbent used for column chromatography was silica gel 60 (70-230 mesh ASTM, E. Merck, Darmstadt). The solvent system used for TLC was CHCl₃-MeOH (5:1 v/v) solution, and the visualization of the TLC plates was performed using a 254 nm UV lamp and I₂ vapor.

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Plant material

The fruits and stems, respectively, of *Opuntia ficus-indica* var. *saboten* Makino (Cactaceae) were obtained in lyophilized powdered form in August 1997 from Cactus Village Processing Center (Pukcheju province), Korea.

Monoamine oxidase (MAO) inhibition assay

The MAO-A activity was determined according to the method previously reported by Ryu *et al.* (1988) using serotonin as a substrate. A reaction mixture containing 0.5 ml of enzyme solution in 10 mM phosphate buffered saline (pH 7.0) and 1 ml of test solution was preincubated at 37°C for 15 min., after which 0.5 ml of 10 mM serotonin creatinine sulfate (Sigma Co.) in a buffer was added. Following incubation at 37°C for 90 min, the enzyme reaction was terminated by heating for 3 min in a 95°C water bath. After centrifugation, 1.6 ml of supernatant was loaded to an Amberlite CG50 (H+ form) column (0.6 cm x 4 cm). The column was washed with over 40 ml of water and the unreacted substrate was eluted with 3 ml of 4 N acetic acid solution and subjected to spectrophotometrical measurement at 277 nm.

Activity was calculated as follows:

\[
\text{Inhibition} \% = \frac{(A_{\text{sample}} - A_{\text{compensate}} - A_{\text{control}})}{(A_{\text{blank}} - A_{\text{control}})} \times 100
\]

The MAO-B activity was also determined according to the method previously reported by Han *et al.*, (1987) using benzylamine hydrochloride as a substrate. The reaction mixture containing 0.5 ml of enzyme solution in the buffer and 1 ml of test solution was preincubated at 37°C for 15 min, after which 0.5 ml of 40 mM benzylamine hydrochloride (Tokyo Kasei Co.) was added. Following incubation at 37°C for 90 min, the enzyme reaction was terminated by adding 0.2 ml of 60% perchloric acid. The reaction product, benzaldehyde, was extracted with 4 ml of cyclohexane and subjected to spectrophotometrical measurement at 242 nm. In the control group, water was substituted for the test solution. In the blank group, the substrate was omitted, but was added after the incubation. To compensate the test solution's own absorbance, the substrate was omitted in the compensate group. Activity was calculated as follows:

\[
\text{Inhibition} \% = \frac{(A_{\text{control}} - A_{\text{sample}} + A_{\text{compensate}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100
\]

Extraction and isolation

The powdered sample of the fruits (4 Kg) was extracted with MeOH (30 L x 4 times) at room temperature for one month. The extract was filtered and the solvent was removed in vacuo. The residue (1.47 kg) was suspended in water 3 L, and partitioned successively with hexane (3 L x 4 times), EtOAc (3 L x 4 times), and BuOH (3 L x 4 times) leaving the water fraction. Each fraction was evaporated in vacuo to yield the residues of a hexane fr. (74 g), a EtOAc fr. (147 g), a BuOH fr. (419 g) and a water fr. (813 g), respectively. The EtOAc fr. showed the strongest MAO-A and -B inhibitory activities in vitro (Fig. 1).

The EtOAc fr. was chromatographed on a silica gel column using stepwise gradient elution with the eluents, CHCl3, CHCl3/MeOH (10:1, 5:1), CHCl3/MeOH/H2O (75:25:2.5, 15:10:2.5). The eluates with CHCl3/MeOH showed the strongest inhibitory activity on MAO-B and were titled a MAO fr.

The MAO fr. (66.2 g) was applied to a silica gel column using stepwise gradient elution with the eluents, CHCl3, CHCl3/MeOH (50:1, 30:1, 10:1, 5:1), CHCl3/MeOH/H2O (75:25:2.5, 15:10:2.5). The subfractions were grouped (fr.1- fr.10) according to their TLC pattern. The subfractions fr.1- fr.6 showed a high MAO-B inhibitory activity on MAO-B (Fig. 3).

Repeated chromatography on silica gel afforded compound I (4.5 g) from fr.1 to fr.6, compound II (0.24 g) and III (0.1 g) from fr.3, and compound (0.05 g) from fr.1.

**Compound I (1,3-dimethyl citrate):** colorless powder; mp. 88-90°C; IR v_max cm⁻¹: 3490, 3430 (OH), 1742 (ester), 1700 (COOH), 1215, 1127, 984 ; El-MS m/z (%): 189 (M+-OCH3, 5), 175 (M+-COOH, 13), 171 (M+-OCH3-H2O, 25), 143 (171-CO, 100); 1H-NMR (CD3OD) δ: 3.66 (6H, s, 2CH3), 2.94 and 2.81 (each 2H, ABq, J= 15.3 Hz, 2 x CH2); 13C-NMR (CD3OD) δ: 176.7 (COOH), 172.2 (COOCH3), 74.3 (C), 52.6 (CH3), 44.2 (CH2)

**Compound II (1-methyl malate):** colorless powder; IR v_max cm⁻¹ : 3443 (OH), 3110 (COOH), 1742, 1711, 1269, 1221, 1182, 1121; 1H-NMR (CD3OD) δ: 4.49 (1H, dd, J=4.7 & 7.2 Hz, CH), 3.73 (3H, s, CH3), 2.77 (1H, dd, J=4.7 & 16.0 Hz), 2.65 (1H, dd, J=7.2 & 16.0 Hz); 13C-NMR (CD3OD) δ: 176.7 (COOH), 172.2 (COOCH3), 74.3 (C), 52.6 (CH3), 44.2 (CH2)

![Fig. 1.](image-url) Monoamine oxidase inhibitory activities of several solvent fractions from the fruits and stems of *Opuntia ficus-indica* var. *saboten*. Each fraction was tested at a concentration of 0.83 mg/ml. A, MAO-A inhibition; B, MAO-B inhibition. □, fruits; ■, stems.